Detection limits in plasmonic whispering gallery mode biosensors

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We analyze a whispering gallery mode biosensor with a metallic nanorod bound to its surface. It is found that a localized surface plasmon resonance in the nanorod can reduce the optical mode volume of the resonator by as much as four orders of magnitude via a local enhancement of the electric field, thus improving the detection sensitivity. Optical frequency shifts as large as 15 MHz are predicted for typical proteins and, for typical experimental parameters, the biosensor is predicted to be limited by laser frequency noise, leading to a minimum detectable polarizability on the order of 10 cubic angstroms.

Whispering gallery mode (WGM) resonators such as silica (SiO_2) microspheres[1] and microtoroids[2] have unprecedented sensitivity as biological sensors[3–6] due to their small optical mode volume and ultra-high quality factor ($Q > 10^8$) in water. The interaction between the resonator's evanescent field and its environment shifts the resonance frequency of the optical mode, such that when a molecule binds to the resonator surface it induces a shift given by[4]

$$\frac{\delta\omega}{\omega} = \frac{-\alpha_m}{2V}\chi_0(\vec{r}_m) \tag{1}$$

where α_m and $\vec{r}_m$ are the polarizability and position of the molecule, $\chi_0(\vec{r}_m) = |E(\vec{r}_m)|^2/\max[|E(\vec{r})|^2]$ with $E(\vec{r})$ being the resonator's electric field, and

$$V = \frac{\int \epsilon_s |E(\vec{r})|^2 dV}{\max[|E(\vec{r})|^2]} \tag{2}$$

is the mode volume of the resonator with relative dielectric permittivity ϵ_s. For typical WGM resonators and proteins, Eq. (1) predicts single molecule optical frequency shifts in the Hz to kHz range. As an example, a bovine serum albumin (BSA; $\alpha_m = 54800$ $\mathring{A}^3$)[7] protein bound to a microtoroidal resonator with $V = 760$ μm^3 and $\chi_0 = 0.4$ produces a frequency shift of 5 kHz. However, measurement noise arising primarily from thermal fluctuations within the resonator and laser frequency fluctuations often limits the minimum detectable frequency shift to above 5 kHz[6,8], precluding the observation of single molecule binding events.

Recently, it was shown that a localized surface plasmon resonance (LSPR) in a large metallic nanoshell bound to the surface of a microsphere resonator can enhance single particle optical frequency shifts by a factor of four[9]. Similarly, in this letter we show that metallic nanorods can enhance the maximum intensity of the electric field by as much as 3×10^4 via a reduction in optical mode volume (via Eq. (2)), producing single molecule frequency shifts on the order of 15 MHz for typical proteins such as

BSA. We analyze the effect of the mode volume enhancement on measurement uncertainties due to thermorefractive noise, frequency noise and phase noise, and find that under typical experimental conditions the minimum detectable polarizability of a bound molecule is reduced to about 10 $\mathring{A}^3$, well below the limit required to measure both single molecule binding events as well as conformational dynamics in some proteins[10].

In this work we have chosen metallic nanorods, as their optical properties have been shown to be highly tunable and largely depend on the nanorod's length-to-diameter aspect ratio. For simplicity it is typical to approximate nanorods as rotational ellipsoids for which two of the principle axes are equal, in which case the nanorod's polarizability is given by[11]

$$\alpha_p = \frac{\epsilon(\omega) - \epsilon_b}{\epsilon_b + (\epsilon(\omega) - \epsilon_b)L}\mathcal{V} \tag{3}$$

where the longitudinal depolarization factor L depends on the length-to-diameter aspect ratio R of the ellipsoid, $\epsilon(\omega)$ is the frequency-dependent dielectric function of the metal, $\mathcal{V} = \pi d^3 R/6$ is the physical volume of an ellipsoid with minor axis d and ϵ_b is the relative permittivity of the background medium. The nanorod exhibits strong resonance when the denominator of Eq. (3) is zero – i.e., when $\epsilon(\omega) = \epsilon_b \frac{L-1}{L}$. Here we consider only the longitudinal dipolar mode of the ellipsoid, for which the depolarization factor is[11]

$$L = \frac{1-e^2}{e^2}\left[\frac{1}{2e}\ln\left(\frac{1-e}{1+e}\right) - 1\right] \tag{4}$$

where the eccentricity e is defined as $\sqrt{1 - R^{-2}}$.

Let us consider a biosensor based on a transverse electric (TE) WGM, with a nanorod bound to the equator so that it experiences the maximum possible electric field, and orientated such that its principle axis is parallel to the surface (Fig. 1A). In this orientation, the nanorod's longitudinal dipole moment is excited by the electric field component parallel with its principle axis, denoted here $E_\parallel(\vec{r}_p) = E_{\text{TE}}(\vec{r}_p)\cos(\phi)$, where E_{TE} is the electric field of the WGM, $\vec{r}_p$ is the nanoparticle's position and ϕ is the angle between the principle axis and the direction of E_{TE} (Fig. 1B). We consider nanorods with dimensions
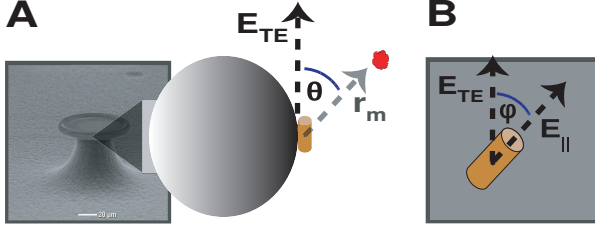
FIG. 1. (A) An SEM image of a 70 μm microtoroidal resonator, and the side view of a gold nanorod at the equatorial surface of the resonator interacting with a target molecule (shown in red). (C) Front view of the nanorod.

much smaller than the incident wavelength λ, so that the external electric field of the nanorod is just that of a radiating dipole[12]

$$E_p(\vec{r} - \vec{r_p}, \theta, \phi) = \frac{(3cos^2\theta - 1)cos(\phi)}{4\pi\epsilon_b r^3}\alpha_p E_{\rm TE}(\vec{r_p}) \quad (5)$$

where $r = |\vec{r} - \vec{r_p}|$ and θ is the angle between the principle axis and the dipole moment of the nanoparticle $\vec{p} = \alpha_p E_{\parallel}(\vec{r_p})$.

The total electric field experienced by a molecule at position $\vec{r_m}$ is then $E(\vec{r_m}) = E_{\rm TE}(\vec{r_m}) + E_p(\vec{r_m} - \vec{r_p})$, with the maximum enhancement in intensity

$$\xi = \max\left[\frac{|E(\vec{r_m})|^2}{|E_{\rm TE}(\vec{r_m})|^2}\right] = \left|1 - \frac{2}{\pi\epsilon_b d^3}\alpha_p\right|^2 \quad (6)$$

occurring at the 'equatorial belt' of the nanorod (where $\phi = 0$, $\theta = \frac{\pi}{2}$, $r = d/2$). From Eq. (2) (see supplemental material[13]) it can be shown that this electric field enhancement reduces the optical mode volume such that

$$V = \frac{V_0}{\xi} \quad (7)$$

where throughout this letter the naught subscript is used to label system parameters prior to the nanoparticle enhancement.

Since the nanorod will strongly scatter and absorb light from the evanescent field near resonance, an additional cavity loss rate Γ must be accounted for, which reduces the optical Q factor of the resonator from Q_0 to $Q = (Q_0^{-1} + \Gamma/\omega)^{-1}$. In the near-field, extinction from the nanoparticle is dominated by absorption[11], with the loss rate determined by $P_{\rm inc}\sigma = \hbar\omega\Gamma$, where $\sigma = 2\pi/\lambda{\rm Im}[\alpha_p]$ is the absorption cross-section[11] and $P_{\rm inc} = \hbar\omega c/V_0$ is the single photon power density incident on the nanoparticle[14], with c being the speed of light. The retardation in Q is

$$\zeta = \frac{Q}{Q_0} = \left(1 + \frac{Q_0}{V_0}2\pi{\rm Im}[\alpha]\right)^{-1} \quad (8)$$

To predict the sensitivity of the WGM-plasmon sensor, we can define the minimum detectable polarizability of a bound molecule as the quadrature sum of uncertainties in the polarizability due to all independent noise sources. In WGM biosensing, these uncertainties most commonly include those which arise from thermofractive noise $\Delta\alpha_T$, laser frequency noise $\Delta\alpha_\Omega$ and phase noise $\Delta\alpha_\Phi$, producing a minimum detectable polarizability of

$$\alpha_{\rm min} = \sqrt{\Delta\alpha_T^2 + \Delta\alpha_\Omega^2 + \Delta\alpha_\Phi^2} \quad (9)$$

These uncertainties are defined in terms of fluctuations in resonator temperature ΔT, laser frequency $\Delta\Omega$ and phase $\Delta\Phi$, respectively, as[13]

$$\Delta\alpha_T = \frac{2}{n}\frac{dn}{dT}V\Delta T(\xi) = \frac{\Delta\alpha_{T,0}}{\xi}\left(\frac{\Delta T(\xi)}{\Delta T_0}\right) \quad (10)$$

$$\Delta\alpha_\Omega = 2V\Delta\Omega = \frac{\Delta\alpha_{\Omega,0}}{\xi} \quad (11)$$

$$\Delta\alpha_\Phi = \frac{V}{Q}\Delta\Phi = \frac{\Delta\alpha_{\Phi,0}}{\xi}\zeta \quad (12)$$

where $n = 1.45$ is the refractive index of SiO$_2$ and dn/dT $= 1.45$ x 10^{-5} K^{-1} is its thermorefractive coefficient.

It is evident from Eqns. (10-12) that the mode volume enhancement affects each noise source differently. In all cases, the measurement uncertainty decreases inversely with the enhancement. The effect is diminished in phase noise due to the retardation in Q, however, as will be shown later, for the experimental regime considered in this letter, fluctuations in phase become negligible when compared to thermal and frequency noise. Importantly, we find that ΔT (thermal fluctuations averaged over the mode volume) depends on the enhancement. Following the analytical work of Gorodetsky and Grudinin[15], we numerically calculated the power spectral density of thermorefractive noise within a typical microsphere resonator as well as the spectrum including the electric field from the nanorod. We then calculated the expected temperature fluctuation ΔT given an averaging time τ of 1 ms, typical for WGM sensing experiments to date[16]. Two important results arose from these calculations. Firstly, we found that, for moderate enhancements ($\xi < 10^3$), the nanorod contributes negligibly to the thermorefractive noise. Secondly, even for enhancements on the order of $\xi \sim 10^5$, the contribution is also negligible if the measurement time τ is much longer than the time scale of the fluctuation across the nanorod ($\sim$100 ps). On the other hand, very large enhancements ($\xi \gg 10^5$) led to significant increases in ΔT, however, this case is uninteresting as it lies beyond the range of enhancements achievable with typical nanoparticles to date[17].

In Fig. 2A and B, we show the mode volume enhancement and Q retardation factors calculated for gold nanorods in water ($\epsilon_b = 1.77$) with diameters $d = 10$ nm and aspect ratios R ranging from 2 to 4. The inital Q factor of the resonator was $Q_0 = 10^7$, and the data for the
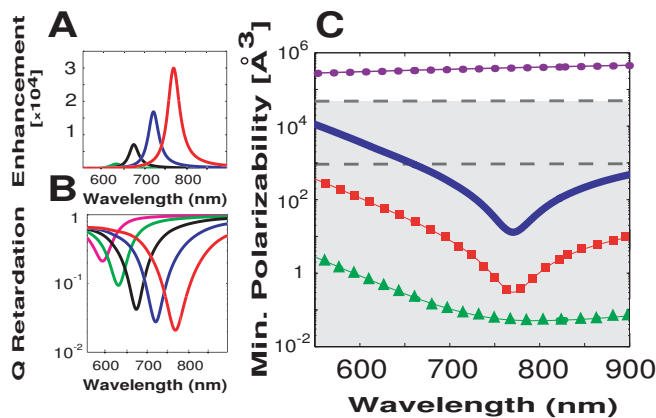
FIG. 2. (A) Mode volume enhancement ξ and (B) retardation in Q. In order of increasing resonant wavelength, data correspond to nanorod aspect ratios of $R = 2, 2.5, 3.5$ and 4. (C) Predicted sensitivity with a nanorod of $R = 4$ (solid), and measurement uncertainties due to thermorefractive noise (squares) and phase noise (triangles). Also shown is the sensitivity of a standard WGM sensor (circles). Top and bottom dotted lines are the polarizabilities of BSA and PYP, and the grey area indicates the region in which single BSA proteins can be detected.

dielectric function of gold was taken from Johnson and Christy[18]. The predicted mode volume enhancement increases for larger aspect ratios, with an enhancement of $\xi = 3 \times 10^4$ for $R = 4$. In this case, the depolarization factor $L = 0.075$ and the LSPR occurs near 770 nm, where the Q factor and mode volume reach minimum values of $Q = 2 \times 10^5$ and $V = 0.025 \ \mu\mathrm{m}^3$, respectively. It is worth noting that, for aspect ratios $R > 4$, the dipole approximation is no longer valid[19], and full-field simulations of the LSPR may be required. Using Eq. (1), we can now estimate the enhancement in optical frequency shifts due to the LSPR. An enhancement of $\xi = 3 \times 10^4$ produces single molecule optical frequency shifts as large as 15 MHz for typical WGM parameters and proteins such as BSA, compared to ~ 5 kHz in standard WGM biosensors. Here, the enhancement is significantly larger than that reported in Ref.[9], on account of the larger LSPR excitations in nanorods.

Shown in Fig. 2C is the predicted minimum detectable polarizability of the WGM-plasmon sensor (solid line). For reference, we have included the sensitivity of a standard WGM sensor (circles) as well as the polarizabilities of a single BSA protein and the photoinduced conformational change of a photoactive yellow protein (PYP) molecule as measured by Stark-effect spectroscopy ($\alpha_{\mathrm{PYP}} = 1000 \mathring{A}^3$)[10] (dashed lines). For the measurement uncertainty due to thermorefractive noise, we found that $\Delta T = 0.6 \pm 0.1 \ \mu\mathrm{K}$ (see supplemental material[13]) for a resonator with radius $\mathcal{R} = 40 \mu\mathrm{m}$ and $\tau = 1\mathrm{ms}$. For frequency noise, it is known that typical diode lasers exhibit frequency fluctuations on the order of the laser's linewidth $\Delta \Omega = 100$ kHz[20]. Finally, consid-

ering an optical power of 1 mW incident on the detector, the fluctuation in phase is $\Delta \Phi = \sqrt{h \omega / (\tau \times 1\mathrm{mW})} \sim 10^{-7}$ radians. In this experimentally realistic regime, with $V_0 = 760 \ \mu\mathrm{m}^3$, frequency noise (solid line) dominates over both thermorefractive noise (squares) and phase noise (triangles), limiting the sensitivity to about $10 \ \mathring{A}^3$ near $\lambda \simeq 770$ nm. This sensitivity is well below the limit required to measure the conformational changes induced in PYP, suggesting that WGM-plasmon sensors could provide a new label-free method of probing conformational changes in small biological machinery such as photosynthetic molecules and molecular motor proteins. Of course, this may require shorter measurement windows (i.e., $\tau < 1$ ms), in which case the measurement uncertainties discussed here would be larger. Interestingly, we also find that the predicted sensitivity of a non-enhanced WGM sensor is larger than the polarizability of BSA.

In summary, we have analyzed the enhancement of a metallic nanorod bound to the surface of a WGM biosensor. The electric field enhancement is significantly larger than that reported previously[9], and the reduction in optical mode volume is predicted to manifest itself in single molecule optical frequency shifts in the MHz range. Equivalently, the enhancement decreases the minimum detectable polarizability of a bound molecule to about 10 $\mathring{A}^3$, highlighting WGM-plasmon sensors as candidates for probing fundamental biophysics on the single molecule level.

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